Cl^- Secretion by Trachea of CFTR (+/−) and (−/−) Fetal Mouse

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The absence of pathologic changes in newborn cystic fibrosis (CF) lung suggests that the fetal CF lung is inflated with a normal volume of liquid and that Cl^- is secreted through paths other than the cystic fibrosis transmembrane conductance regulator (CFTR)-associated Cl^- channel. We studied liquid content of distal lung and transepithelial electrical potential difference (PD) of cultured cystic tracheal explants from 16 to 19 day gestation fetal mice of CFTR (+/−) (heterozygous) females that were mated with CFTR (−/−) "knockout" males. Distal lung water content was not affected by fetal genotype. Basal PDs were not different (CFTR (+/−), 8.6 mV, and CFTR (−/−), 9.1 mV), and PDs of both groups were inhibited by intraluminal injection of amiloride (10^{-4} M) (−25%) and after addition of bumetanide (10^{-4} M) to the bath (−40%). Terbutaline (3×10^{-5} M) induced a similar increase in PD (about 65%) in both groups. Intraluminal injection of ionomycin (2×10^{-5} and 5×10^{-6} M) raised PD in both groups (CFTR (+/−) by 32 and 27% and CFTR (−/−) by 41 and 11%). All of the increase in PD induced by terbutaline and ionomycin was inhibited by bumetanide. The PD response to terbutaline was not attenuated by pretreatment with ionomycin or the Ca^{2+} chelator BAPTA (10^{-4} M). Ionomycin or ATP, but not terbutaline, increased intracellular Ca^{2+} concentration of isolated cultured tracheal epithelial cells. The response of CF epithelium to β-adrenergic agonist suggests that cyclic AMP-activated Cl^- pathways other than those linked to CFTR regulate liquid secretion in fetal mouse airways.

Our understanding of the mechanisms that control Cl^- secretion in the fetal lung (1) is incomplete. Cystic fibrosis transmembrane conductance regulator (CFTR) is thought to function as a cyclic AMP (cAMP)-regulated Cl^- channel (2). The presence of abundant CFTR in fetal airways (3) suggests that this protein may have an important role in liquid secretion, but the absence of functional CFTR protein in CF fetuses does not appear to impede secretion of lung liquid. An alternative, Ca^{2+}-activated Cl^--secretory pathway has been shown in the respiratory epithelia of both fetal and postnatal human lung (4, 5). This path can be stimulated by inflammatory mediators and purinergic agents (4–6) and is the focus of therapeutic strategies to overcome defective Cl^- secretion in CF patients (7).

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Abbreviations: cyclic AMP, cAMP; Ca^{2+} concentration, $[Ca^{2+}]$; cystic fibrosis, CF; cystic fibrosis transmembrane conductance regulator, CFTR; polymerase chain reaction, PCR; transepithelial electrical potential difference, PD.

Agents that raise cell cAMP concentration induce Cl^- secretion by airway epithelia of adult mammalian lung (8–10) and Na^+ absorption by alveolar or whole lung preparations (11, 12). The action of cAMP in the fetal lung is more complex. cAMP has a stimulatory effect on liquid and Cl^- secretion by both proximal and distal regions of excised or cultured immature fetal lung (4, 8–10, 13–16). However, late in gestation, secretion of liquid by the lung *in vivo* is inhibited or switched to absorption by infusion of epinephrine into fetal sheep (17), an effect that is mediated by Na^+ absorption in the distal lung. Initiation of absorption helps to clear liquid from the lumen at the time of birth and prepares the fetal lung for air breathing. There is no evidence that this adaptive response is abnormal in neonates with cystic fibrosis (CF).

Development of a transgenic mouse model of CF by gene deletion (18) provides an opportunity to study the reliance of fetal liquid secretion on paths of ion permeation linked to CFTR. In this study, we compared the basal secretion of lung liquid by distal lung by measuring the water content of lungs excised from CF (CFTR −/−) and heterozygote (CFTR +/−) late-gestation fetal mice (term = 19 days). We also measured the transepithelial electrical potential difference (PD) across cultured cystic explants derived from fetal trachea. We evaluated the relative importance of secretory paths that are activated by second messengers from voltage changes induced by agents that raise intracellular cAMP or

Ca^{2+}. We also measured intracellular Ca^{2+} concentration ($[Ca^{2+}]$) to assess possible cross-activation of the Ca^{2+}-sensitive pathway by terbutaline, a β-adrenergic agonist and a traditional activator of the cAMP-mediated path.

Materials and Methods

Fetal mice were produced by mating males homozygous for CFTR gene deletion, CFTR (−/−), with females who were heterozygous carriers of the defect (CFTR (+/−)). In addition, we studied fetal lungs from two litters that were the product of CFTR (+/−) mothers mated with CFTR (+/+) fathers. Fetuses were removed after the dams were killed by CO_2 inhalation.

Wet:Dry Weight Measurements

Fetal lungs with trachea from three litters were excised, and the trachea was clamped. The lungs were blotted with premoistened filter paper, and the lobes were separated from the main bronchi and dropped into a tared flask. The flasks with tissue were weighed wet and dry (> 14 h at 80°C) as described previously (19).

Explant Culture

Tracheas were explanted into submersion culture as described for trachea and distal lung of fetal rat (13). Briefly, tracheas were excised and placed in an isosmotic collagen-salt solution that gelled after incubation at 37°C. Explants submerged in the gel were bathed in F12 supplemented with 10% fetal bovine serum, 10 mM HEPES (pH 7.4), and 2 mM $NaHCO_3$. Explants were cultured at 37°C in a 95% air–5% CO_2 environment for 7 days. Culture medium was changed every 48 h.

Basal PD and the Effects of Drugs on PD

After 7 days in culture, PD was measured across the walls of all explants that formed liquid-filled cysts. Cysts were impaled with a glass microelectrode filled with 3 M KCl (tip resistance ranged from 5 to 40 MΩ) as described previously (13). The electrode was connected through an Ag-AgCl half cell to a high impedance voltmeter and referenced to a bridge (3% agar in bath medium) in the bath through a calomel half cell. PD was measured continuously through a period of basal PD measurement and during changes induced by drugs that were added to the cyst lumina or bath.

Terbutaline sulfate (3×10^{-3} M in saline; Geigy, Ardsley, NY) or bumetanide (10^{-1} M in dimethyl sulfoxide; Sigma Chemical Co., St. Louis, MO) was added to the bath to give a final concentration of 3×10^{-5} or 10^{-4} M, respectively. When drugs that act from the lumen were tested, a measured volume of vehicle (with or without drug) was microinjected into the cyst lumen with a glass pipette with a bevelled tip (diameter = 5 to 15 μm [13]). The injected bolus was equivalent to 1% of average cyst volume. Consequently, stock solutions of amiloride HCl (10^{-2} M in water; gift from Merck Sharp and Dohme, West Point, PA) and Ca ionomycin (5×10^{-4} or 2×10^{-3} M in 10 to 20% ethanol; Boehringer Mannheim, St. Louis, MO) were expected to be diluted to 10^{-4} M and 5×10^{-6} M or 2×10^{-5} M, respectively. Impalement by the injection pipette caused a transient change in PD (mean = −10%, range = +10% to −45% change from the stable baseline [defined as < 0.5 mV change/2 min]). All drug responses are reported as the first stable values after microinjection or exposure to drug in the bath. One-hour control experiments with continuous impalement with a recording electrode showed a small decline in basal PD over this time (Table 1A).

Genotype Determination

Genomic DNA was prepared from the tail and one leg of each fetus by the salting-out method adapted from Miller and colleagues (20). DNA from each fetus was amplified by two different polymerase chain reactions (PCR). One set of primers is specific for wild-type DNA and yields a PCR product (identified by gel electrophoresis) only when wild-type DNA is present. The second primer set is specific for targeted (disrupted) CFTR DNA and yields a PCR product only when targeted DNA is present. DNA from a heterozygous fetus yields a PCR product from both PCR, whereas DNA from a homozygous CF fetus yields only the targeted DNA product. Both PCR amplifications were repeated to verify CF genotype. Genotype was determined 2 to 3 wk after removal of the fetus from the dam so that the investigators were unaware of the origin of the tissue during studies of function.

Calcium Measurements

Individual fetal tracheal epithelial cells were isolated by incubation of fetal trachea with protease 0.1% in minimal essential medium for 2 h at 37°C and cultured in F12 plus 10% fetal bovine serum for 18 to 36 h on vitrogen-coated coverslips. Cells were loaded with Fura-2/AM, and fluorescence was induced and measured with a modular fluorimeter system (Fluorolog 2, model F2C; Spex Industries, Edison, NJ) attached to an inverted microscope (Nikon) as described previously (21).

Statistics

Differences between basal PDs and PDs after exposure to drugs were compared by the paired t test. When more than one drug was added during the experiment, we adjusted the t value for significance (0.05) with the Bonferroni correction. Differences between lung weights, basal PD, or drug-induced PD changes for explants with different genotypes were compared by the Mann-Whitney U test. Percentage changes in PD are normalized to the basal PD.

Results

Effect of Genotype on Fetal and Lung Growth

Timed pregnancies of heterozygote (CFTR (+/−)) females mated with homozygous "knockout" males without CFTR (CFTR (−/−)) produced 20 litters, with an average litter size of 8.9 ± 0.6 fetuses. Litter sizes of two pregnancies in which the father was CFTR (+/+) were 5 and 8 fetuses. There was no trend between the proportion of CF (−/−) in a litter and litter size. Eighty of a total of 177 fetuses from CFTR (+/−) and CFTR (−/−) mating had a CFTR (−/−) genotype (46%).

Fetal weights, dry lung weights, and water weight/dry lung weight ratios were not different for CFTR (+/−) and CFTR (−/−) fetuses from three litters measured at 16, 17, and 18 days of gestation (Figure 1).

TABLE 1

*A. Control data**

	Δ PD (*mV*)	Δ Basal PD (%)	Time Elapsed (*min*)	*n*
Time control	−0.8 ± 1.1	−11	50	3
Saline injection	−0.1 ± 0.2	−2	5	5

B. Effect of drugs†*

Drug Added	Pretreatment	CFTR	Δ PD (*mV*)	Δ Basal PD (%)	*n*
Amiloride (10^{-4} M)	None	+/−	−2.1 ± 0.2	−25	58
		−/−	−2.9 ± 0.3	−29	26
		+/+	−1.6 ± 0.5	−19	5
Bumetanide (10^{-4} M)	None	+/−	−2.1 ± 0.4	−35	4
		−/−	−2.4 ± 0.4	−42	4
BAPTA (10^{-4} M)	None	+/−	−6.2 ± 1.1	−33	4
		−/−	−2.6 ± 1.3	−20	4
Terbutaline (3×10^{-5} M)	None	+/−	6.1 ± 0.4	67	11
		−/−	4.8 ± 0.6	65	12
	Amiloride	+/−	5.8 ± 0.4	59	14
		−/−	5.2 ± 0.7	61	8
		+/+	4.6 ± 0.7	60	6
	Amiloride/ionomycin (5×10^{-6} M)	+/−	4.2 ± 0.5	60	21
		−/−	3.6 ± 0.9	34	6
	Amiloride/ionomycin (2×10^{-5} M)	+/−	1.8 ± 0.6	33	8
		−/−	1.7 ± 0.6	29	5
	BAPTA	+/−	7.1 ± 0.5	63	5
		−/−	4.6 ± 1.2	67	3
Ionomycin (5×10^{-6} M)	Amiloride	+/−	2.2 ± 0.3	27	21
		−/−	1.2 ± 0.3	11	6
Ionomycin (2×10^{-5} M)	Amiloride	+/−	2.4 ± 0.7	32	8
		−/−	2.7 ± 0.6	41	5
Ionomycin (5×10^{-6} M)	Amiloride/terbutaline	+/−	0.5 ± 0.1	6	14
		−/−	1.1 ± 0.3	13	8
		+/+	1.2 ± 0.3	15	5

* All values are mean ± SEM.

† Δ PD values are significantly different from pretreatment values except for changes in PD after ionomycin, following pretreatment with amiloride and terbutaline.

Tracheal Explants

In submersion culture, the cut ends of tracheas sealed over within 48 h in 91% CFTR (+/−) explants and 95% CFTR (−/−) explants, resulting in the formation of cystic structures by liquid secretion into the lumen. After 7 days in culture, the tracheal epithelium of explants from both genotypes (CFTR (+/−) and CFTR (−/−)) was well differentiated, with a mix of ciliated and nonciliated cells (Figure 2).

Transepithelial PD

After 7 to 9 days in culture, mean basal PDs of CFTR (+/+) (7.2 ± 0.7, n = 12), CFTR (+/−) (8.6 ± 0.4 mV, n = 87), and CFTR (−/−) (9.1 ± 0.6 mV, n = 50) explants were not different.

The relative contributions to total resting PD of Cl^- secretion and Na^+ absorption were evaluated from the response to the Na^+/Cl^- cotransport blocker bumetanide in the bathing solution and amiloride injection into the cyst lumen. Bumetanide caused a gradual decline of PD over 60 min to 65% of basal values for CFTR (+/−) and 58% of basal PD for CFTR (−/−) (Table 1B). Resting PD was inhibited within 30 s of amiloride injection. By steady state (5 min after injection), the change in PD from basal values across CFTR (+/−) explants (−25%) was similar to that of CFTR (−/−) explants (−29%) injected with amiloride (Table 1B). Injection of an equivalent volume of saline (7 nl) did not induce a significant change in PD over the same time (Table 1A).

Explants were exposed to a selective β_2-adrenergic agonist, terbutaline, and to the Ca^{2+} ionophore ionomycin to

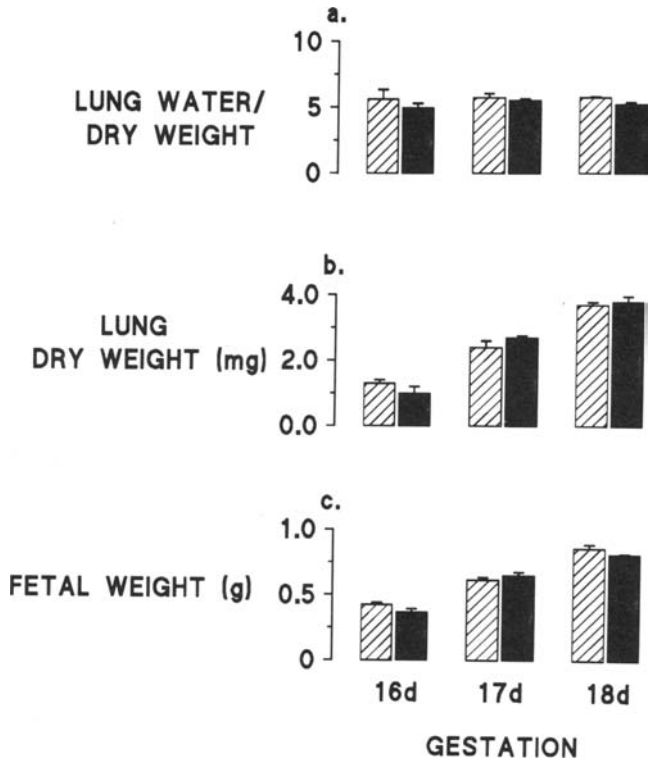

Figure 1. Gravimetric analysis of CFTR (+/−) (*shaded bars*) and CFTR (−/−) (*closed bars*) fetuses at 16, 17, and 18 days of gestation. a. Mean lung water/dry weight ± SEM. b. Mean lung dry weight ± SEM. c. Mean fetal weight ± SEM.

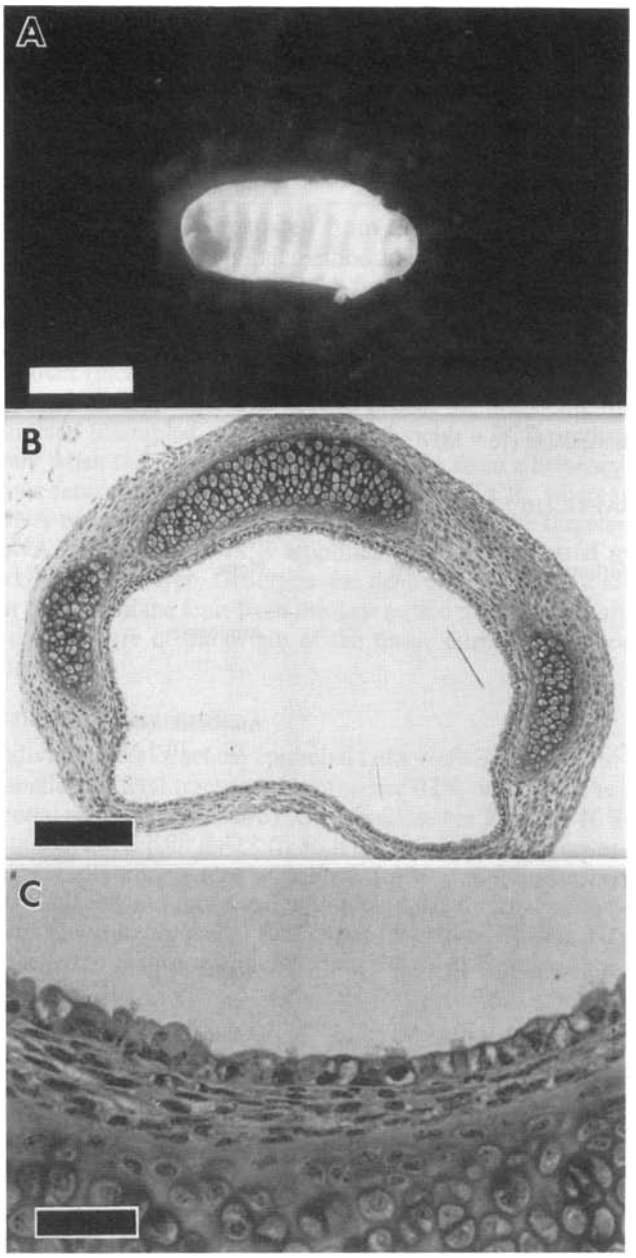

Figure 2. Cystic tracheal explant from 19 day gestation CFTR (−/−) fetus after 7 days in culture. A. Low-power light micrograph of explant in submersion culture shows cut ends sealed over and tracheal rings well preserved. Bar = 500 μm. B. Cross-section of fixed explant, stained with Richardson stain. Bar = 60 μm. C. High-power view of cross-section of fixed explant shows a mix of ciliated and nonciliated epithelial cells, with underlying layers of connective tissue cells and cartilage. Bar = 15 μm.

test for cAMP- and Ca^{2+}-mediated changes in PD. Terbutaline raised basal PD by 65 to 67% in CFTR (+/−) and CFTR (−/−) explants (Table 1B). Pretreatment with amiloride did not affect stimulation of PD across CFTR (+/−) and CFTR (−/−) cysts by terbutaline (Table 1B).

Ionomycin was added to the bath or injected into cysts that had been pretreated with amiloride. Addition of ionomycin to the bath tended to increase PD slightly, but this change was not significant. The higher dose of ionomycin (2×10^{-5} M) resulted in frequent pipette plugging, short-lived formation of precipitate within the cyst, and a significantly greater increase in the PD of CFTR (−/−) explants (2.7 mV) compared with the lower dose (1.2 mV) (Table 1B). The magnitude of responses to ionomycin for CFTR (+/−) explants was the same for both doses (Table 1B).

After the response to ionomycin had stabilized, terbutaline (10^{-5} M) was added to the basolateral bath. The absolute increase in PD induced by subsequent addition of terbutaline was not different for explants pretreated with 5×10^{-6} M ionomycin, but the percentage increase for the CFTR (−/−) group (34%) tended to be less than that for the CFTR (+/−) group (60%) because the basal PD of the CFTR (−/−) group was greater. The fractional contribution of the terbutaline-stimulated PD to the total change in PD induced by terbutaline and ionomycin was, however, the same for both groups (0.73 in CFTR (+/−) and 0.70 in CFTR (−/−)). Pretreatment with 2×10^{-5} M ionomycin resulted in similar increases in PD when explants of both genotypes were exposed to terbutaline, but these responses were significantly smaller than those for explants that were not pretreated with ionomycin.

When the order of drug addition was reversed and ionomycin (5×10^{-6} M) was injected into CFTR (+/−)

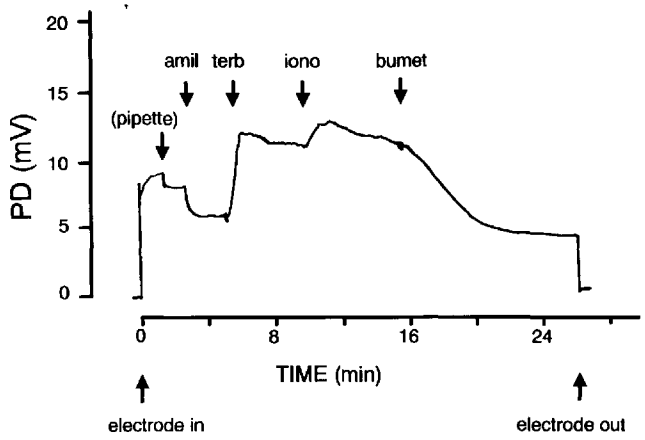

Figure 3. Time course of transepithelial PD across tracheal explant from 18 day gestation CFTR (+/−) fetus. Upward arrows show insertion into and withdrawal of the microelectrode from the cyst lumen. First downward arrow denotes impalement of the cyst with a micropipette. Subsequent downward arrows show times of amiloride (10^{-4} M) microinjection, terbutaline (5×10^{-5} M) addition to the bath, ionomycin (5×10^{-6} M) microinjection, and bumetanide (10^{-4} M) addition to the bath.

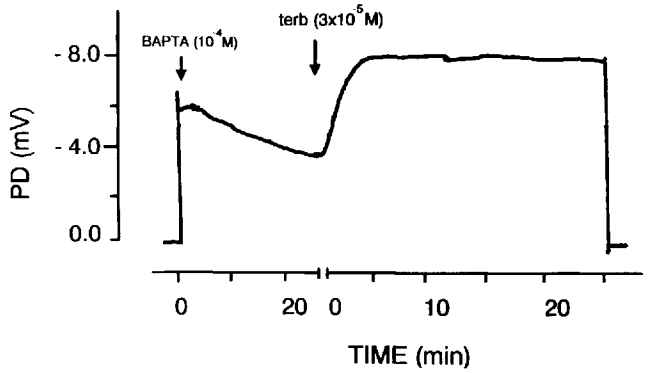

Figure 4. Time course of transepithelial PD across tracheal explant from 17 day gestation CFTR (−/−) fetus. BAPTA (10^{-4} M) was added to the bath. After 40 min, terbutaline (5×10^{-5} M) was added to the bath. Note the change in time scale after addition of terbutaline.

explants that had been exposed to amiloride and terbutaline, the hyperpolarization response was smaller than with explants pretreated with amiloride alone (Table 1B). The ionomycin response of CFTR (−/−) explants pretreated with terbutaline was not attenuated (Figure 3). Bumetanide (10^{-4} M) was added to the bath when PD stabilized after addition of ionomycin and terbutaline (usually about 6 min after addition of terbutaline). We observed an abrupt inhibition of stimulated PD (Figure 3) that stabilized within 10 to 20 min (CFTR (+/−), $-102 \pm 9\%$, $n = 11$; CFTR (−/−), $-110 \pm 8\%$, $n = 4$). Without bumetanide, PD increased slowly by an additional $23 \pm 6\%$ ($n = 6$) between 6 and 14 min after addition of terbutaline.

Cell Calcium and the Terbutaline Response

Activation of a Ca^{2+}-dependent path by the β-adrenergic agonist was tested by pretreating explants with an intracellular Ca^{2+} chelator, BAPTA (10^{-4} M in the bath), for 45 min before addition of terbutaline (3×10^{-5} M) to the bath (Figure 4). BAPTA caused a fall in basal PD of 33% in CFTR (+/−) and 20% in CFTR (−/−). Subsequent exposure to terbutaline induced the same increase in PD in both groups (Table 1B). The magnitude of the change was similar to that induced by terbutaline alone.

We also measured changes in intracellular $[Ca^{2+}]$ (Fura-2 fluorescence) induced by terbutaline, forskolin, ionomycin, and ATP in ciliated and nonciliated tracheal epithelial cells (from 6 fetuses: 2 CFTR (−/−), 1 CFTR (+/−), and 3 CFTR (+/+)) that were cultured for 24 to 48 h on vitrogen-coated coverslips. Terbutaline (5×10^{-5} M) or forskolin (10^{-5} M) induced no change in baseline intracellular $[Ca^{2+}]$. Ionomycin (5×10^{-6} M) or ATP (10^{-4} M) caused a transient 5- to 14-fold increase in $[Ca^{2+}]$ followed by a plateau that was about twice the basal $[Ca^{2+}]$ (Figure 5). We detected no clear difference in the pattern of response between ciliated and nonciliated cells or between different genotypes.

Discussion

Our study shows that fetal growth, lung growth, and water content of distal lungs excised from heterozygote and "knockout" CF fetal mice late in gestation were not different. We also found that, in general, PD responses of tracheal explants from the three genotypes (CFTR (+/+), CFTR (+/−), and CFTR (−/−)) to drugs that affect Na^+ or Cl^- flux across respiratory epithelia were not different.

The proportion of CF (−/−) fetuses per litter (0.46) was close to that predicted from parental genotype (0.5). A trend towards a lower-than-expected proportion of CF fetuses was noted at all litter sizes. If confirmed by studies of larger populations, this finding would be more likely to result from problems with conception (e.g., a smaller frequency of viable CFTR (−) eggs or sperm), rather than from fetal death.

The similarity between voltage changes induced by β-adrenergic agonists in CFTR (−/−) and CFTR (+/−) explants contrasts with the minimal bioelectric response to cAMP in explants and monolayers cultured from human fetal distal CF lung (15, 22). Although basal PD and liquid secretion

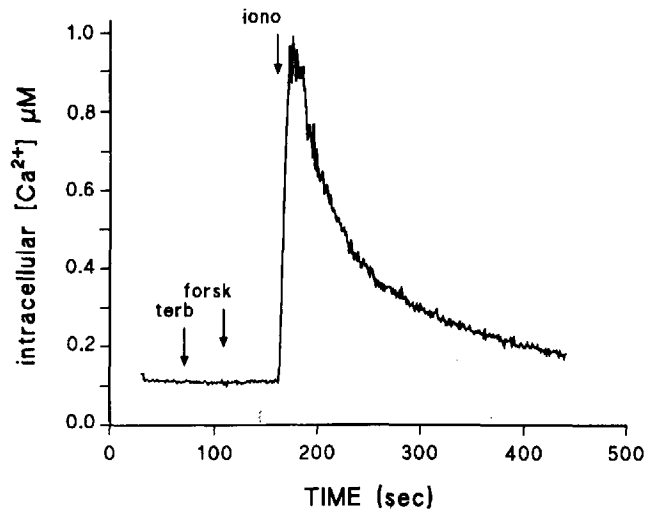

Figure 5. Time course of fluorometric analysis of intracellular $[Ca^{2+}]$ in ciliated tracheal epithelial cell from CFTR (+/−) fetus.

were reported to be similar in normal and CF human fetal explants, stimulation of PD by adrenergic agonists was detected only in non-CF explants. The differences in response to cAMP in the midtrimester CF human fetal distal lung compared with response in the late-gestation fetal mouse trachea may relate to gestational and regional differences in the tissues used in these studies. This possibility draws some support from studies of CFTR expression in normal human fetal lung which show that CFTR expression diminishes in tracheal epithelium in late gestation while persisting at higher levels in small airways (3). Our attempts to study the bioelectric properties of cultured explants from the distal mouse lung were not successful because of poor cyst formation. Alternatively, the minimal bioelectric responses to cAMP in the human CF fetal studies may indicate a greater importance of CFTR-linked Cl^- path in human fetal lung than in mouse fetal lung.

The hyperpolarization we observed after terbutaline and ionomycin exposure is likely to reflect stimulation of Cl^- secretion since preparations had been pretreated with amiloride, and Cl^- and Na^+ are thought to be the principal ions transported across the fetal pulmonary epithelium. The rapid inhibition of this hyperpolarization by bumetanide is further evidence that Cl^- secretion was stimulated by ionomycin and terbutaline. We sought to explain the apparent Cl^--secretory response to β-adrenergic agonist, the hallmark defect of postnatal CF airways epithelia, by the trachea of CF fetal mouse. First, terbutaline could raise intracellular $[Ca^{2+}]$ and activate Cl^- channels not associated with CFTR. Interaction of cAMP with Ca^{2+}-activated channels in CF tracheas seemed unlikely because the absolute increase in PD induced by doses of ionomycin that are close to the limit of solubility was smaller than that induced by a maximal dose of terbutaline. More direct evidence against mediation of the terbutaline response by Ca^{2+} is provided by the absence of an inhibitory effect of BAPTA on the terbutaline response and by the absence of any measurable change in intracellular $[Ca^{2+}]$ after terbutaline or forskolin in isolated tracheal epithelial cells.

There was, however, evidence for interaction between the two drugs at some point in the stimulation-effect sequence because pretreatment with one agonist sometimes blunted the subsequent response to the other. The trend was, however, not restricted to CFTR (−/−) explants (Table 1B).

The best-described example of cAMP activation of Ca^{2+}-dependent channels is the excised trachea of the adult mouse described by Grubb and co-workers (23). This preparation is characterized by a maximal cAMP stimulation of short-circuit current that is substantially smaller than that induced by ionomycin. In addition, the response to cAMP is blocked by BAPTA and paralleled by a clearcut increase in $[Ca^{2+}]$ of tracheal epithelial cells. By contrast, fetal tracheal explants respond more vigorously to β-adrenergic agonist than to ionomycin. Although they are depolarized by BAPTA, fetal preparations share neither BAPTA inhibition of the cAMP response nor changes in cell $[Ca^{2+}]$ with the tracheal epithelium of the adult. Fetal and postnatal $[Ca^{2+}]$ were measured in similar preparations (i.e., recently isolated single cells) and gave different results, but the possibility exists that cAMP may induce a rise in $[Ca^{2+}]$ in confluent fetal epithelial barriers. In addition, pretreatment of postnatal mouse trachea with ionomycin eliminated the forskolin response, whereas similar pretreatment of fetal mouse tracheal explants with ionomycin had no effect on the subsequent depolorization response to terbutaline. Consequently, the pattern of interaction between terbutaline and ionomycin we described above is more likely to result from voltage change limited by the establishment of a dominant permeability (e.g., P_{Cl^-} of the apical membrane and/or P_{K^+} of the basolateral membrane) than by mediation of the effect through a common Cl^- channel. Accordingly, we conclude that there is minimal crossover of the cAMP message to Ca^{2+}-activated channels in fetal tracheal explants from both genotypes.

The Cl^--secretory responses of tracheal preparations from postnatal mouse (5, 23) suggest that the Cl^--secretory response of airway epithelium to cAMP diminishes after birth while responsiveness to intracellular $[Ca^{2+}]$ increases. A similar reciprocal switch from dominant cAMP- to dominant $[Ca^{2+}]$-activated cell volume regulation and Cl^- efflux has been described for distal fetal rat lung epithelial cells in the last few days of gestation (24). In that study, cAMP effects were inhibited by incubation with antisense oligodeoxynucleotides to the human CFTR gene. These observations suggest that a decline in CFTR mRNA may account for the emergence of a dominant Ca^{2+}-activated Cl^- conductance in specific regions of the postnatal lung.

The brisk bioelectric responses of fetal CF mouse tracheal explants to terbutaline contrasted with the relatively small (compared with ionomycin) responses to forskolin in freshly excised trachea from both normal and adult CF mice (23). When postnatal tracheal epithelial cells were cultured on collagen membranes, however, forskolin induced a small increase in short-circuit current across preparations from normal but not CF mice (5).

The lack of any detectable differences in distal lung water content or dry lung weights between CFTR (+/−) and CFTR (−/−) explants suggests that fetal lung liquid production and lung growth is not affected in the CF fetus, and is concordant with the observation that lungs from CF human fetuses are normal at birth. The response of the CF fetal tracheal explants to terbutaline raises the possibility that cAMP-activated Cl^- channels, other than those associated with CFTR, exist in the developing fetal mouse trachea. Non-CFTR cAMP-sensitive Cl^- channels have not, as yet, been described.

The fetal CF lung may be protected from the consequences of abnormal Cl^- transport for other reasons. Our study shows that a Ca^{2+}-activated Cl^--secretory pathway, similar to those described for human fetal distal lung (4) and a variety of postnatal epithelia, is present in tracheal epithelium from both CFTR (+/−) and CFTR (−/−) fetuses, and this path may protect fetuses with abnormal CFTR. The observation that baseline liquid secretion and basal PD of cultured distal lung explants from human CF fetuses was not compromised (15) suggests that CFTR-linked Cl^- transport may not be required for basal liquid secretion in the fetal lung. This possibility is supported by *in vivo* studies of fetal sheep lung which show that epinephrine *decreases* lung liquid secretion (17) during the latter part of gestation. Consequently, β-adrenergic agonist stimulation of secretion by air-

ways of the mature fetus is overridden by events in the distal lung where liquid is absorbed in response to the same β-adrenergic stimulus. Because amiloride inhibition of the PD was small and not different for tracheas explanted from fetuses of both genotypes, we conclude that fetal CF airway epithelium is not characterized by hyperabsorption of Na^+ that defines CF large airway epithelia in postnatal life (25). Basal liquid secretion by the fetal pulmonary epithelium may be adequate for lung development in the CF fetus but insufficient to hydrate the CF airway when hyperabsorptive forces are recruited in postnatal life.

In addition to ion transport mechanisms, mechanical factors might protect the CF fetal lung from the effects of liquid hyposecretion. Normal lung growth is dependent on the maintenance of the developing lungs in a state of balanced expansion, so that underinflation or overexpansion of the lung *in utero* leads to lung hypoplasia or hypertrophy, respectively (26). But, even if the liquid secretion rate in the fetal CF lung is diminished, autoregulation of fetal lung volume and hydrostatic pressure by control of liquid efflux (27, 28) may allow the CF fetal lung to expand sufficiently for normal development.

Our study points to the possible existence of cAMP-activated Cl^- channels that are not linked to CFTR and provides a rationale for the observation that the lungs of newborn infants with CF develop normally. Further study of these fetal lung secretory mechanisms may lead to better understanding of the physiologic role of CFTR in the fetal lung and novel approaches to the treatment of CF in the postnatal lung.

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